

## EDITORIAL COMMENT

# MicroRNA Augmentation of Bone Marrow-Derived Cell Therapy\*

Stefan Engelhardt, MD, PhD,<sup>†‡</sup> Yassine Sassi, PhD<sup>†</sup>

The means to regenerate cardiac muscle that has been lost due to myocardial infarction (MI) or cardiomyopathies continues to be a central focus of cardiovascular research. A large spectrum of methodologies and approaches has been developed and tested in experimental models, with an increasing number undergoing testing in clinical trials (1).

Next to approaches that directly target those cells remaining after a myocardial insult, the exogenous delivery of cells to injured myocardium has received much attention (2). The spectrum of cells experimentally delivered for myocardial regeneration is immense and includes skeletal myoblasts, various myocardial cell populations (e.g., cardiospheres, c-kit<sup>+</sup> cells), and cardiac myocytes derived from pluripotent and induced pluripotent stem cells (1,2). Many of these approaches share the limitations that they are complex, technically demanding, and difficult to standardize. The clinical testing is mostly at an early stage, with considerable safety issues to be resolved.

In contrast, the isolation and transplantation of bone marrow-derived mononuclear cells (BMMNCs) are routine in the clinical setting for several hematologic conditions and have been increasingly applied for nonhematologic diseases, with a major focus on cardiovascular indications. A simplistic review of the

current trial results is that this therapeutic modality appears to be largely safe, with efficacy ranging between modest and absent (3). This notion holds true also for studies using not unfractionated BMMNCs but subsets of these cells that express certain markers like CD34, such as utilized by Joladarashi et al. (4) in this issue of the *Journal*. Some researchers regard this cell population as an equivalent of the much-noticed endothelial progenitor cells (EPCs). However, there is no universally accepted definition of EPCs, and several more complex sets of markers are in use (5).

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Mechanistically, a consensus is forming that the various transplanted BMMNC preparations do not survive, differentiate, or fuse to an extent that reaches functional relevance but rather seem to act through paracrine factors (6). Taken together, the transplantation of BMMNCs appears feasible and safe, yet an increase in their therapeutic efficacy would be highly desirable and may even be indispensable for further clinical development of this therapeutic modality.

It is against this background that Joladarashi et al. (4) manipulated the levels of an endogenous micro-ribonucleic acid (miRNA) as a means to increase the therapeutic efficacy of transplanted CD34<sup>+</sup> cells for cardiac repair. As a starting point for their study, the investigators sought to identify miRNAs that are functionally relevant in these cells. To do so, they treated CD34<sup>+</sup> cells with an inflammatory stimulus (lipopolysaccharide [LPS], an activator of innate immune signaling) known to activate these cells, and found that miR-377 is downregulated. Transfection of synthetic miR-377 inhibited the proangiogenic proteome that these cells secrete, making miR-377 a candidate molecule to optimize CD34<sup>+</sup> cell-based therapies. A second interesting observation was the prominent

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From the <sup>†</sup>Institut für Pharmakologie und Toxikologie, Technische Universität München, Munich, Germany; and the <sup>‡</sup>German Center for Cardiovascular Research (DZHK), partner site Munich Heart Alliance, Munich, Germany. Drs. Engelhardt and Sassi are supported in part by the German Federal Ministry of Education and Research in the context of the e:Med program (e:AtheroSysMed) and the Deutsches Zentrum für Herz-Kreislauf-Forschung.

upregulation of miR-377 in the myocardium of failing hearts. Angiogenesis may aid revascularization of ischemic myocardium, and the researchers therefore speculated that inhibition of anti-angiogenic miR-377 in CD34<sup>+</sup> cells before transplantation may enhance their therapeutic effect. Indeed, the study reported higher capillary densities when ischemic mice received CD34<sup>+</sup> cells pre-treated with inhibitors against miR-377. Most importantly, mice treated with miR-377-depleted CD34<sup>+</sup> cells showed an improvement in cardiac function and myocardial remodeling. The authors conclude that inhibition of miR-377 is a promising strategy to enhance the therapeutic efficacy of CD34<sup>+</sup> cell transplants in ischemic cardiac damage.

The present study closely matches a recent study by Wen et al. (7) that likewise used knockdown of miR-377 in transplanted cells (mesenchymal stem cells [MSCs]) to enhance their therapeutic efficacy upon transplant in a model of MI. Similar to the present study, Wen et al. reported remarkable increases in vascular and capillary densities and functional protection 4 weeks after MI and concomitant injection

of miR-377-depleted MSCs. Mechanistically, Wen et al. (7) found that miR-377 directly targets vascular endothelial growth factor (VEGF) and (at least in vitro) the antiangiogenic properties of miR-377 are mediated through repression of VEGF. Thus, although both groups studied a different cell population, they both report that miR-377 inhibited the proangiogenic properties of these cells.

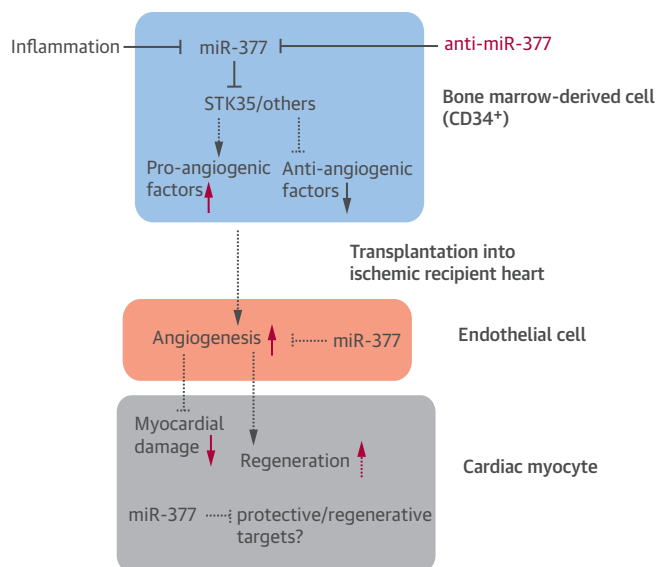
It is natural that a complex experimental setting such as the transplantation of bone marrow-derived cells into a model of myocardial ischemia also evokes a number of questions. Before we can regard miR-377 as a regulator of cardiac regeneration, several issues need to be addressed (see dotted arrows and symbols in Figure 1) that mainly refer to the proposed mechanism of action.

What is the pathophysiological role of the increase in miR-377 levels in diseased myocardium? Given the relatively large increase in miR-377 levels in the myocardium post-MI and the fact that this occurred in all cell types studied by Joladarashi et al. (4), miR-377 also becomes an obvious candidate to control angiogenic factors such as VEGF from the myocardial side. If this is the case, inhibition of miR-377 within the host myocardium may confer therapeutic efficacy in its own right.

What are the direct target molecules of miR-377 in CD34<sup>+</sup> cells? The researchers identified a single direct target, which is serine/threonine kinase 35 (STK35). Until now, the function of STK35 in biology and disease has remained largely enigmatic. Whether and how this kinase regulates the behavior of transplanted CD34<sup>+</sup> cells is an interesting question that needs further investigation. At the same time, miR-377 likely targets a number of alternative molecules shaping the angiogenic profile of these and possibly other cells.

What are the downstream secreted factors, with alterations upon depletion of miR-377 that are responsible for the proangiogenic effect of these engineered cells? Transfection of miR-377 repressed a number of proangiogenic proteins (VEGF, hepatocyte growth factor, angiopoietin, and others) and inhibited antiangiogenic factors (thrombospondin-1, platelet factor-4, and others), but inhibition of miR-377 yielded only subtle changes compared with control treatment conditions. This may be explained by low levels of miR-377 in the cells investigated yet is surprising in light of the strong proangiogenic effects observed in vivo. Which of these factors alone or in combination are ultimately responsible for the proangiogenic signal elicited from transplanted miR-377-manipulated CD34<sup>+</sup> cells is unresolved and will require additional studies. From another

**FIGURE 1** Suggested Mechanism of Action for miR-377 in CD34<sup>+</sup> Cells



MicroRNA-377 (miR-377) targets serine/threonine kinase 35 (STK35) and possibly other molecules in bone marrow-derived mononuclear cells positive for CD34. As a net effect, miR-377 inhibits the release of a proangiogenic secretome, inhibiting angiogenesis and possibly cardiac regeneration. Therapeutic inhibition of miR-377 enhances the angiogenic properties of CD34<sup>+</sup> cells. Experimentally verified mechanisms and changes are depicted with **solid lines** and putative mechanisms and changes with **dotted lines**. **Red** symbolizes changes observed with anti-miR-377 treatment.

perspective, these molecules may likewise represent therapeutic targets themselves.

What happens on the recipient side (i.e., the injured myocardium) that benefits from the depletion of miR-377 in the transplanted cells? The investigators reported an increase in capillary density in line with angiogenic effects upon inhibition of miR-377 in human umbilical vein endothelial cells. Although the latter experiment yielded significant increases in migration and tube formation of these cells, we can only speculate whether this effect mirrors what would be seen after the addition of media from miR-377-depleted CD34<sup>+</sup> cells. This would be important to know because a consensus has emerged in the field that these and similar cells form at best very few endothelial cells and rather exert their effects through paracrine signals (5,6). An alternative mechanism of action may be related to the very acute phase of cell loss, where the CD34<sup>+</sup> cells were applied and where they may affect cell loss. This would be in line with the considerable (yet nonsignificant) trend toward smaller infarcts and with what Wen et al. (7) showed for transplantation of miR-377-depleted MSCs in an MI model of permanent left anterior descending artery ligation (7).

Eventually, a direct comparison of the various means proposed to enhance the functional capacity of transplanted cells in well-controlled models is desirable, as is the identification of the preferred cell type. Given the overwhelming complexity of the mechanisms involved, a systematic analysis of this kind would be difficult to conduct, however. What we can realistically hope for are smaller side-to-side comparisons of some of the most efficacious strategies developed to date that may help to better focus on those types of cell manipulation that have a realistic chance to succeed also in clinical trials.

The present study is timely because the field is eagerly awaiting the results of the large phase III BAMI (Bone Marrow-Derived Mononuclear Cells on All-Cause Mortality in Myocardial Infarction; NCT01569178) clinical trial. If this clinical trial turns out positively, approaches such as the one developed by Joladarashi et al. (4) will have additional relevance to the field.

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**REPRINT REQUESTS AND CORRESPONDENCE:** Dr. Stefan Engelhardt, Institut für Pharmakologie und Toxikologie, Technische Universität München, Biedersteiner Straße 29, Munich 80802, Germany. E-mail: [stefan.engelhardt@tum.de](mailto:stefan.engelhardt@tum.de).

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